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(57) Abstract

The invention relates to a lipid particle forming matrix, characterized by, that from a defined system of at least two lipid components chosen from classes of different polarity, in which at least one of the lipid components is bilayer forming, discrete lipid particles are formed spontaneously when interacting with aqueous systems. Preferably at least one of the lipid components is amphiphatic and polar and one is nonpolar. These discrete particles are formed spontaneously from the matrix without any chemical or physical treatment or initiation. The lipid particle forming matrix can contain bioactive materials, chosen from the group of drugs, herbicides, pesticides, fertilizers, food and cosmetic ingredients or additives. The invention also relates to a process for the production of the matrix, the use of the lipid particle forming matrix as a carrier system for bioactive materials and pharmaceutical composition such as oral, rectal, nasal, vaginal, ocular or parenteral vehicles, creams, ointments, capsules and tablets containing the said lipid particle forming matrix and a drug.

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LIPID FORMULATION SYSTEM

INTRODUCTION

The present invention relates to lipid matrices which provide the release of bioactive agents through the formation of a type of liposomes *in vivo* when the matrices interact with water. The spherical lipid bilayers thus formed *in vivo*, hereinafter referred to as Biosomes (or lipid particle) and the lipid matrix, referred to as a Biosome Forming Matrix (BFM), should be separated from the well established concept of liposomes or liposome technology which are defined as the formation of lipid vesicles in an aqueous phase or in a freeze-dried form already prepared *in vitro* before administration. The invention also relates to the production and use of these lipid matrices (BFM).

BACKGROUND

Parenteral depot systems are widely known to those skilled in the art and are well accepted concepts for long term delivery of drugs. These systems are based on biodegradable polymer systems or lipid formulations, e.g. oil solutions and oil suspensions. However, both systems show a serious disadvantage since, after the drug release process has terminated, the lipids or polymer carriers are still at the injection site for a long period of time and, for some systems such as implants, they may even have to be eliminated by surgery. Furthermore, the application of either oils or biodegradable polymers such as polylactic/polyglycolic acid show limited applications since each concept requires specific physicochemical properties of the bioactive material to be included into the systems, e.g. solubility or stability/compatibility.

Hence, parenteral therapy needs a delivery system for bioactive materials applicable for both highly polar as well as nonpolar bioactive materials for which the delivery system shows an intrinsic rate controlling mechanism for drug release, which can be varied over an extensive time frame. A characteristic for such delivery system should be that both the drug release and the biodegradation occur simultaneously.

Since parenteral administration of bioactive materials often needs to be carried out by physicians or nurses and the fact that many people find such therapy uncomfortable, a lot of effort is made on developing drug delivery

forms applicable for other routes of administration. Still, the most common route of administration is the enteral (oral, rectal) but during the past decade several attempts have been made to develop intranasal or transdermal delivery systems as alternatives to the parenteral route.

However, the adsorption through biological membranes is a very complex process due to the varying nature of the different membranes to be bypassed as well as the varying nature of the bioactive material used. Many enterally administered drugs also show a high biotransformation when absorbed from the gastrointestinal tract or show a restricted or erratic absorption capacity due to their physicochemical properties, molecular size or sensitivity to degradation processes in the gut, or due to some specific absorption mechanism in limited parts of the gastrointestinal tract. Also, bioactive material administered intranasally or dermally may show erratic and irregular absorption and many delivery formulations hence need the addition of absorption enhancers which in some cases have been shown to be detrimental to the nasal mucosa or the skin due to local side effects.

Due to this lack of regularity, the enteral/nasal/dermal therapy needs a delivery system which eliminates this variability and which is sufficiently flexible for incorporating a variety of bioactive materials, independent of their physicochemical properties, molecular size or source of origin, particularly for such bioactive materials which currently cannot be administered via the enteral route due to limited absorption capacity.

Several papers have been published demonstrating the influence of lipids on drug absorption. However, various results have been obtained showing an enhanced oral absorption either in man or animals, for example:

- griseofulvin in an oil-in-water emulsion (Bates and Sequeria, J. Pharm. Sci., **1975**, *64*, 793),
- cefoxitin in an oil-in-water emulsion (Palin et al., Int. J. Pharm., **1986**, *33*, 99),
- Insulin in liposomes of phosphatidylcholine/cholesterol, as well as in water-in-oil microemulsion (Patel and Ryman, FEBS Letters, **1976**, *62*, 60; Cho and Flynn, Lancet, **1989**, Dec. 23/30),
- cyclosporine in microemulsion (Tarr and Yalkowsky, Pharm. Res. **1989**, *6*, 40),

- enhanced nasal absorption in rats of insulin in solution with lysophosphatidylcholine (Illum et al., Int. J. Pharm., **1989**, *57*, 49).

Decreased absorption was found for propranolol in coconut oil (Palin et al., J. Pharm. Pharmacol., **1989**, *41*, 579) or no effect at all for vitamin K incorporated into mixed micelles based upon glycolate and lecithin (Winn et al., J. Pharm. Pharmacol., **1989**, *41*, 257). Furthermore, Rowland and Woodley (Biochim. Biophys. Acta, **1980**, *620*, 400) have shown that many liposomal systems are quite unstable in the gastrointestinal tract and that drugs incorporated into liposomes gave the same absorption compared to free drug *per se*. It has recently been indicated in thermodynamic studies, that human insulin-DEAE-dextran complex entrapped in liposomes may present a more stable system than the uncomplexed and/or unentrapped human insulin. However, no evidence that this really works *in vivo* have been shown (Manosroi et al., Drug Dev. Ind. Pharm., **1990**, *16*, 837).

In some cases there is a therapeutic need to administer bioactive materials locally, such as in wounds after surgery or for the treatment of burns. In those cases, a need exists to deliver the bioactive material locally as well as for an extended period of time in a controllable manner since after surgery no further administration of the formulation is possible, and as in the case of burn injuries, pain may cause severe discomfort to the patient upon repeated administrations. Furthermore, local application to other regions in the body, such as in the vagina, with an extended drug delivery may show therapeutic advantages.

It is well known to those skilled in the art that bioactive materials can be entrapped into unique lipid/aqueous spherical structures defined as liposomes. A liposome is defined as a structure consisting of one or more concentric spheres of lipid bilayers separated by water or aqueous buffer compartments. Thus far, liposome formation and hence manufacturing, has been restricted to techniques where the said formation is carried out *in vitro*.

Numerous patents and scientific papers on liposomes have been published and the technical field of applying various lipid derivatives in combination with amphiphatic compounds such as phospholipids are well known to those skilled in the art. Liposomes can be prepared by different methods using solvents, reduced pressure, two-phase systems, freeze drying, sonication etc.

(Weiner et al., Drug Dev. Ind. Pharm. **1989**, *15*, 1523). The process technology assigned to these methods is highly complicated. Due to the specific demand in terms of the physicochemical properties of the drug molecule in order to form stable liposome structures, only a limited number of candidate drugs have been shown to be applicable in liposomes formed *in vitro*. The major application of liposomes have so far been restricted to parenteral delivery and for cosmetic skin care products even though attempts have been made for other routes of administration such as oral, nasal, pulmonary. The applications for parenteral use have been focused on intravenous administration and drug targeting and to some minor extent for extended or controlled release from a depot. Thus far, the applications of liposomes are restricted to the formation and incorporation of bioactive materials *in vitro*.

A composition for oral delivery of drugs has been disclosed in a patent by Yesair (WO 86/05694), comprising non-esterified fatty acids, monoglycerides with fatty acids having 14-18 carbon atoms, lysophosphatidylcholine in which the fatty acid component has 14-18 carbon atoms and a drug. None of these single-chained components are bilayer-forming which is a prerequisite for at least one of the lipid components in the present invention.

US Patent 4,610,868 discloses a way of producing liposomes where water-soluble compounds are incorporated. However, this patent deals with globular structures present from the beginning, in contrast to the present invention. The said invention also uses organic solvents in the process which is in contrast to the present invention where the Biosomes are formed spontaneously without any chemical or physical treatment or initiation.

Other documents disclosing the preparation of liposomes are EP 158 441, EP 260 241 and WO 87/07502. According to EP 158 441, in contrast to the present invention, at least one water-miscible liquid (e.g. glycerol, ethanol) and 5-40 % water should be added to at least one membrane lipid (e.g. phospholipids such as soy lecithin and egg yolk lecithin. EP 260 241 discloses a dry lipid-based solid material which forms or reconstitutes liposomes in the presence of water. This composition should be dehydrated, e.g. through lyophilization or spray-drying which should not destroy the liposome structure. The liposome structure is thus present from the beginning, in contrast to the present invention. WO 87/07502 discloses a pro-liposome formulation comprising of at least one volatile liquid propellant and at least

one lipid component. Also in this case discrete particles are formed by dehydration and thus the liposomes are present from the beginning.

The current well known liposome technology, where the systems are prepared *in vitro* before administration, suffers from the disadvantage that the systems are quite unstable and factors such as temperature or other constituents present in the formulation may dramatically change the nature of the liposomes by irreversibly damaging the bilayers. It is also well known (see Weiner above) that liposomes composed of crude egg yolk phosphatides are not physically stable *in vitro* at ambient temperatures for more than a few months which limits the application of these formulations in routine practice. By applying the matrix according to the invention the above mentioned stability problems can be avoided.

The above mentioned problems and needs can be met by using a delivery system as described in this application. The present invention, relating to Biosome formation *in vivo*, will show advantages as compared to already well known lipid drug delivery systems.

The present invention discloses a way to produce, use and/or utilize an entrapment or adsorption procedure for bioactive substances into unique lipid matrices. Such a combination may be used as a pharmaceutical formulation within human and veterinary medicine, in agriculture, or as cosmetic or food/nutritional formulations.

Figure 1 shows a microscope photo of the formulation according to Example 9. Figure 2 shows a microscope photo of the formulation according to Example 10.

DESCRIPTION OF THE INVENTION

According to the invention a lipid particle forming matrix is characterized by that from a system of at least two defined lipid components chosen from classes of different polarity, in which at least one of the lipid components is bilayer forming, discrete lipid particles are formed spontaneously when interacting with excess aqueous systems. A defined lipid component is a lipid whose chemical composition is known and controlled. In the system at least one of the lipid components is amphiphatic and polar and one is nonpolar.

The amphiphatic and polar component is preferably phosphatidylcholine and the nonpolar lipid is preferably chosen from the classes of mono-, di- and triglycerides or a mixture thereof. At room temperature the lipid particle forming matrix has a liquid or semi-solid consistency.

The amount of the polar lipid class components should be in the range of 5-80 % (w/w) of the lipid system, preferably in the range of 10-60 % (w/w).

The amount of the polar and amphiphatic lipid class components should be in the range of 5-80 % (w/w) of the lipid system, preferably in the range of 25-50 % (w/w).

Preferably the lipid particle forming matrix contains bioactive materials, which could be chosen from the groups of drugs, herbicides, pesticides, fertilizers, food and cosmetic ingredients or additives. The amount of bioactive material is below 70 % (w/w) of the matrix, preferably below 50 % (w/w).

In the lipid particle forming matrix the discrete particles are formed spontaneously from the matrix without any chemical or physical treatment or initiation.

When preparing the lipid particle forming matrix the amphiphatic and polar or the nonpolar lipid is mixed with the bioactive material *per se*, or in solution, and preferably the nonpolar lipid or lipids are admixed to the mixture of the bioactive material and the amphiphatic and polar lipid or lipids.

The lipid particle forming matrix could be used as a carrier system for bioactive materials and especially in pharmaceutical compositions such as oral, rectal, nasal, vaginal, ocular or parenteral vehicles, creams, ointments, capsules and tablets and they could be used for the manufacturing of a pharmaceutical composition for enteral, parenteral, nasal, intravaginal, ocular administration or administration locally on skin, wounds or mucous membranes.

The property 'bilayer forming' is a well-known physical parameter and can easily be established by suitable physicochemical methods (e.g. surface balance method). The establishment of the formed discrete lipid particles can be done by physical and/or chemical methods, such as microscopy using polarized light, or diffraction methods.

The present invention relates to bioactive materials to be entrapped in lipid matrices and will not be restricted to any particular class of bioactive material in terms of physicochemical properties, molecular size or the source of origin, i.e. synthetic, biotechnological materials, etc. The variation in the lipid composition provides the control mechanism by which Biosomes are formed and thereby to the rate of Biosome formation which will serve as a controlling factor for either immediate or sustained release of the entrapped or associated bioactive materials.

The matrix of the invention can only be defined in general terms as given in Claim 1. The difference between the matrix according to the invention and already known lipid systems, is the capability of spontaneous formation of the Biosomes in contact with excess aqueous media. Thus, by *a)* using well defined lipid components from at least two different lipid classes and by *b)* designing these lipid components into unique lipid matrices which form Biosomes *in vivo* when interacting with water, the system according to the invention can be obtained.

A bioactive material, within the scope of the present invention, is defined in its broadest sense, such as a biologically active substance having effect and/or is used within human and/or veterinary medicine, cosmetics as well as within agricultural areas (pesticides, herbicides and/or fertilizers). Also included are areas such as food.

Any type of bioactive agent can be applied. Hence, this invention is focused on the principle of lipid particle forming matrices which may contain a bioactive agent where said bioactive agent and hence the Biosome forming matrix design are based upon the physicochemical properties of the various matrix components.

For the person skilled in the art it is obvious that these substances are not by any means limited to the use within the areas mentioned above, the substances can be, and are used for other purposes or indications than the ones described above. Furthermore, in human and veterinary medicine a pharmacologically active substance, a salt, solvate, enantiomer, or a polymorph thereof may be used, including substances that are synthetic or biosynthetic in their origin. In agricultural areas substances that are used as herbicides or substances that act as stimulators on crop may be used. Also substances that

have an effect on various parasites (pesticides) are included. Within the food area the invention may be used to incorporate additives, such as vitamins, preservatives, spices or other taste-carriers in order to protect and/or release such substances in connection with consumption or storage of food.

The following definitions are used:
lipids - a general term for natural or synthetic compounds consisting of acyl carriers, such as glycerol, sphingosine, cholesterol, and others or derivatives thereof, to which one or more fatty acids are or could be linked. Also similar molecules that contains a substantial hydrocarbon portion may be included.

The lipids used for the Biosome Forming Matrices (BFM) can be classified into different lipid classes depending on their polarity, namely:

nonpolar lipid classes - these have no polar head groups. Examples of nonpolar constituents are hydrocarbons, or non-swelling amphiphiles, such as mono-, di- and triacylglycerols, cholesterol, fatty alcohols or cholesterol esters.

polar lipid classes - these have polar head groups and possess surface activity, such as phospholipids or glycolipids. Depending on their specific interactions with water they are further subdivided into the categories of swelling and soluble amphiphiles.

amphiphatic or amphiphilic lipid classes - such as phospholipids and glycolipids, being surface active.

bilayer forming lipid classes - amphiphatic lipids, such as PC (phosphatidylcholine), sphingomyelin, PI (phosphatidylinositol), with a molecular geometry that preferentially leads to bilayer structures in the presence of water.

The lipids used for the BFM consist of a mixture of lipid classes characterized by their different polarities. Polar lipids, such as phospholipids or glycolipids, and nonpolar lipids, such as mono-, di- and triglycerides, are the main constituents in the system but also sterols, such as cholesterol, fatty acids, fatty alcohols and esters thereof as well as other lipid classes may be utilized. This well defined mixture of lipids from different classes as defined above, should not be confused with commercial products such as soybean oil, maize oil or soy lecithin and egg lecithin. To get the well defined lipid classes the

commercial material, such as an oil or a lecithin, is fractionated and then the different lipid classes are admixed as explained in more detail in the examples below.

Furthermore, derivatives of lipids may also be used in combination with the above mentioned lipids. One example of this is polyethyleneglycol coupled to phospatidylethanolamine, which has shown to prolong the circulation time of liposomes after injection in the blood stream. Another example of such a derivative is palmitoylcarnitine which acts as an absorption enhancer for bioactive substances in the gut.

In the preferred way of initiating the formation of the BFM, the bioactive substance is admixed to a selected lipid, followed by admixing of a lipid of a different polarity. This polar/nonpolar alteration may be continued for as many cycles as necessary in the specific case, involving a range of lipids with different polarities.

The preferred way of incorporation of a bioactive substance into the BFM is to admix the bioactive substance to amphiphilic lipids in order to create a homogeneous formulation, where the amount of amphiphilic lipids generally is in the total range of 5-80 % (w/w). Such an amphiphilic lipid should be capable of spontaneous bilayer formation. Examples thereof are amphiphilic and polar lipid classes, such as phosphatidylcholine, phosphatidylglycerol, phosphatidylinositol or phosphatidylserine or mixtures thereof.

In order to prevent or delay an immediate interaction of the amphiphile(s) with exogenous water, the BFM should also contain one or more nonpolar lipid class. Examples of such nonpolar lipids are mono-, di- or triglycerols, cholesterol or its esters.

Endogenous water, ethanol or other solvents may be present in small amounts (not enough for Biosome formation) in the BFM if the bioactive substance needs such a solvate to be incorporated.

The design of the BFM includes not only the proper selection and/or combination of lipid classes, tailor-made for the solubilization of each bioactive substance, but also the proper selection of the distribution of fatty acids, i.e. the acyl groups attached to the utilized lipid classes. Variation of the acyl groups

gives different physicochemical properties as will be seen in the examples below.

By varying the geometrical shape of the main bilayer forming lipid class, i.e. the effective head group area in relation to the steric conformation of the hydrocarbon tails, the rate by which the Biosomes are formed from the BFM in a given aqueous environment can be affected and controlled.

A second way of affecting and controlling the formation of Biosomes is by varying the structure, thus the fluidity, of the hydrocarbon chains in the nonpolar part of the BFM. This will affect the rate of interaction of the endogenous amphiphatic lipids and the exogenous aqueous medium.

Thus, a careful selection of lipid constituents for a specific BFM will be required in order to *a)* incorporate the bioactive compound *in vitro* and to *b)* release the bioactive component by Biosome formation *in vivo*. This involves the selection of lipid classes as well as the distribution of the fatty acid residues and therefore requires access to analytically pure and well-characterized lipids. The examples below will further illustrate the variation of the matrices by selection of lipids and combinations thereof without limiting the scope of invention.

Thus, the invention relates particularly to the design and behaviour of the BFM which is a new concept for drug delivery of bioactive materials. The invention does not restrict the application of the BFM to any specific route of administration since the BFM will show potential applications for a variety of drug delivery forms such as absorbtion enhancement of oral, rectal, nasal, dermal formulations or controlled delivery via the parenteral route or locally, e.g. in the vagina or in wounds.

After the formation of the Biosomes *in vivo*, by means of a controllable rate, drug molecules entrapped into, or associated to the BFM are rapidly liberated once the Biosomes appear in the blood circulation in order for the drug to be able to act pharmacologically. This assumption is supported by the fact that liposomal structures are known to interact rapidly with plasma proteins such as albumin, transferin and macroglobulins, but are also hydrolyzed *in vivo* by specific phospholipases (Wiener et al., Drug Dev. Ind. Pharm., **1989**, *15*,

1523). Thus, the use of mortar compounds may be omitted according to the present invention.

According to the present invention, it is possible to incorporate both highly polar as well as nonpolar bioactive materials, in a flexible manner, into a lipid matrix structure by means of a combination of nonpolar lipids and amphiphatic compounds and that these drug containing BFM's form Biosomes, when the BFM's interact with water, thus generating a drug delivery system suitable for either an enhanced or controlled extravascular absorption or a controlled parenteral drug release combined with a biodegradation.

The present invention provides an improved and flexible drug delivery system applicable for various classes of bioactive materials. *In vitro* release experiments of vitamin B12 (cyanocobolamine) have shown that it is possible to obtain BFM's with different Biosome forming rates as a function of the BFM composition. Furthermore, parenteral drug delivery with controlled release has been found even for highly water-soluble bioactive material such as fragmentated heparin (Fragmin®) using the present invention. Such a combination of a highly hydrophilic bioactive substance with a hydrophobic carrier has hitherto not yet been shown. To those skilled in the art this new and unique property of the present lipid drug delivery carrier (i.e. BFM) must be regarded as highly unpredictable. The results which confirm this are shown in Examples 9, 10 and 15. Also, it has been shown to be possible to incorporate a synthetic low molecular weight substance, i.e. buspirone (cf. Example 22) as well as a high molecular weight compound, i.e. coenzyme Q10 (cf. Example 23).

By incorporating bioactive materials according to the lipid matrix principle in this invention, referred to as Biosome Forming Matrices, the following advantages are obtained compared to conventional pharmaceutical dosage forms, or delivery systems:

- a drug delivery system consisting of a lipid matrix and a bioactive material which can be designed in a flexible manner showing a unique capacity for incorporation of either polar or nonpolar bioactive materials showing a wide range of molecular weights without changing the chemical structure and hence the biological activity of the materials.

- a drug delivery system consisting of BFM and a bioactive material which forms Biosomes *in vivo* and for which the rate of Biosome formation can be altered by unique combinations of nonpolar and amphiphatic lipids derivatives.

- a drug delivery system consisting of a lipid matrix and a bioactive material which can be used for multipurpose applications such as extravascular absorption enhancement, parenteral controlled drug delivery or local extended drug delivery for which each specific purpose can be achieved by unique lipid combinations in a flexible manner.

- a drug delivery system consisting of a lipid matrix and bioactive material which is thermodynamically stable.

- a drug delivery system in which the drug and the carrier simultaneously are degraded.

- a drug delivery system which gives a possibility to improve the oral administration of high molecular weight compounds such as proteins, peptides, polysaccharides, etc.

This invention relates solely to the concept and design of the novel lipid matrices, the Biosome Forming Matrices, which show a unique formation of Biosomes *in vivo* after administration, and in which any suitable bioactive material can be incorporated, if needed for any particular reason, such as for improved bioavailability or for extended/controlled release purposes.

Various modifications and equivalents will be apparent to the one skilled in the art and may be used in the compounds, compositions and methods of the present invention without departing from the spirit or scope thereof, and it is therefore to be understood that the invention is not to be limited to the specific examples and embodiments herein.

EXAMPLES

EXAMPLE 1

1.25 g phospholipid from soybean (I) is added to 1.25 g of a glyceride mixture (II) and gently stirred for 12 h at 60 °C. 2.50 g of a triglyceride (III) is then added and the total mixture is stirred for 1 h at 60 °C.

Lipid class composition (g)	I	II	III	Fatty acid composition of triacylglycerol (wt%)	
Phosphatidylcholine	0.50				
Phosphatidylethanolamine	0.40				
Phosphatidylinositol	0.23				
Nonpolar lipids	0.12				
Monoacylglycerol		0.63			
Diacylglycerol		0.63			
Triacylglycerol			2.50		
				8:0 caprylate	*58.5*
				10:0 caprate	*40.5*
				12:0 laurate	*0.6*
				minors	*0.4*
Total	1.25	1.25	2.50	*Total*	*100*

EXAMPLE 2

1.25 g phospholipid from soybean (I) is added to 1.25 g of a glyceride mixture (II) and gently stirred for 12 h at 60 °C. 2.50 g of a triglyceride (III) is then added and the total mixture is stirred for 1 h at 60 °C.

Lipid class composition (g)	I	II	III	Fatty acid composition of triacylglycerol (wt%)	
Phosphatidylcholine	0.40				
Phosphatidylethanolamine	0.35				
Phosphatidylinositol	0.18				
Phosphatidic acid	0.07				
Nonpolar lipids	0.25				
Monoacylglycerol		0.63			
Diacylglycerol		0.63			
Triacylglycerol			2.50		
				8:0 caprylate	*58.5*
				10:0 caprate	*40.5*
				12:0 laurate	*0.6*
				minors	*0.4*
Total	1.25	1.25	2.50	*Total*	*100*

EXAMPLE 3

1.25 g phospholipids from soybean (I) is added to 1.25 g of a glyceride mixture (II) and gently stirred for 12 h at 60 °C. 2.50 g of a triglyceride (III) is then added and the total mixture was stirred for 1 h at 60 °C.

Lipid class composition (g)	I	II	III	*Fatty acid composition of triacylglycerol (wt%)*	
Phosphatidylcholine	0.50				
Phosphatidylethanolamine	0.40				
Phosphatidylinositol	0.23				
Nonpolar lipids	0.12				
Monoacylglycerol		0.63			
Diacylglycerol		0.63			
Triacylglycerol			2.50		
				16:0 palmitate	*10.0*
				18:0 sterarate	*2.8*
				18:1 oleate	*20.6*
				18:2 linoleate	*58.9*
				18:3 linolenate	*6.7*
				minors	*1.0*
Total	1.25	1.25	2.50	*Total*	*100*

EXAMPLE 4

1.25 g phospholipid from soybean (I) is added to 1.25 g of a glyceride mixture (II) and gently stirred for 12 h at 60 °C. 2.50 g of a triglyceride (III) is then added and the total mixture was stirred for 1 h at 60 °C.

Lipid class composition (g)	I	II	III	*Fatty acid composition of triacylglycerol (wt%)*	
Phosphatidylcholine	0.40				
Phosphatidylethanolamine	0.35				
Phosphatidylinositol	0.18				
Phosphatidic acid	0.07				
Nonpolar lipids	0.25				
Monoacylglycerol		0.63			
Diacylglycerol		0.63			
Triacylglycerol			2.50		
				16:0 palmitate	*10.0*
				18:0 sterarate	*2.8*
				18:1 oleate	*20.6*
				18:2 linoleate	*58.9*
				18:3 linolenate	*6.7*
				minors	*1.0*
Total	1.25	1.25	2.50	*Total*	*100*

EXAMPLE 5

1.25 g phospholipid from soybean (I) is added to 1.25 g of a glyceride mixture (II) and gently stirred for 12 h at 60 °C.

Lipid class composition (g)	I	II
Phosphatidylcholine	0.40	
Phosphatidylethanolamine	0.35	
Phosphatidylinositol	0.18	
Phosphatidic acid	0.07	
Nonpolar lipids	0.25	
Monoacylglycerol		0.63
Diacylglycerol		0.63
Triacylglycerol		
Total	1.25	1.25

EXAMPLE 6

1.25 g phospholipid from soybean (I) is added to 1.25 g of a glyceride mixture (II) and 0.16 g ethanol. The total mixture is gently stirred for 6 h at 60 °C. 0.16 g of a triglyceride (III) is added and the total mixture is stirred for another hour at the elevated temperature.

Lipid class composition (g)	I	II	III
Phosphatidylcholine	0.40		
Phosphatidylethanolamine	0.35		
Phosphatidylinositol	0.18		
Phosphatidic acid	0.07		
Nonpolar lipids	0.25		
Monoacylglycerol		0.63	
Diacylglycerol		0.63	
Triacylglycerol			0.16
Total	1.25	1.25	0.16

EXAMPLE 7

15 mg cyanocobalamin (B12) is added to 1.25 g of a glyceride mixture (II) and gently stirred for 3 h at 60 °C. 1.25 g phosphatidylcholine from soybean (I) is added and the stirring continues for 6 h at 60 °C. 2.50 g of a triglyceride (III) is added and the total mixture is stirred for another hour at the elevated temperature.

Lipid class composition (g)	I	II	III	*Fatty acid composition of triacylglycerol (wt%)*	
Phosphatidylcholine	1.25				
Monoacylglycerol		0.63			
Diacylglycerol		0.63			
Triacylglycerol			2.50		
				8:0 caprylate	*58.5*
				10:0 caprate	*40.5*
				12:0 laurate	*0.6*
				minors	*0.4*
Total	1.25	1.25	2.50	*Total*	*100*

EXAMPLE 8

15 mg cyanocobalamin (B12) is added to 1.25 g of a glyceride mixture (II) and gently stirred for 3 h at 60 °C. 1.25 g phosphatidylcholine from soybean (I) is added and the stirring continues for 6 h at 60 °C. 2.50 g of a triglyceride (III) is added and the total mixture is stirred for another hour at the elevated temperature.

Lipid class composition (g)	I	II	III	*Fatty acid composition of triacylglycerol (wt%)*	
Phosphatidylcholine	1.25				
Monoacylglycerol		0.63			
Diacylglycerol		0.63			
Triacylglycerol			2.50		
				16:0 palmitate	*10.0*
				18:0 sterarate	*2.8*
				18:1 oleate	*20.6*
				18:2 linoleate	*58.9*
				18:3 linolenate	*6.7*
				minors	*1.0*
Total	1.25	1.25	2.50	*Total*	*100*

Below in Table I, viscosity, melting temperature and melting enthalpy have been measured for the compositions according to Examples 1-8.

Table I

Example	Viscosity (mPa s)	T_m (°C)	ΔH (J/g)
1	167	–	–
2	104	-37.9 - 3.5	5.9 50.3
3	199	-72.1 -23.1	0.7 41.9
4	104	-72.1 -22.2	0.7 42.0
5	2100	-17.2	18.7
6	–	-26.1 + 6.4	36.5 14.4
7	133		
8	900		

Viscosity measured on a Bohlin VOR rheometer at 25 °C. T_m (phase transition temperature) and ΔH (enthalpy change at transition) obtained by means of differential scanning calorimetry.

As can be seen in Table I, various physicochemical properties can be obtained for the BMF's as a function of the lipid combinations used as well as the fatty acid compositions. This will enable the manufacturing of BMF's showing a wide variety of physical properties.

EXAMPLE 9

30 mg cyanocobalamin (B12) is added to 2.50 g of a monoglyceride (II) and the mixture is gently stirred for 3 h at 60 °C. 2.50 g phosphatidylcholine from soybean (I) is added and the stirring continues for 6 h at 60 °C. 5.00 g of a triglyceride (III) is added and the total mixture is stirred for another hour at the elevated temperature.

Lipid class composition (g)	I	II	III	*Fatty acid composition of triacylglycerol (wt%)*	
Phosphatidylcholine	2.50				
Monoacylglycerol		2.50			
Triacylglycerol			5.00		
				8:0 caprylate	*58.5*
				10:0 caprate	*40.5*
				12:0 laurate	*0.6*
				minors	*0.4*
Total	2.50	2.50	5.00	*Total*	*100*

EXAMPLE 10

30 mg cyanocobalamin (B12) is added to 2.50 g of a monoglyceride (II) and the mixture is gently stirred for 3 h at 60 °C. 2.50 g phosphatidylcholine from soybean (I) is added and the stirring continues for 6 h at 60 °C. 5.00 g of a triglyceride (III) is added and the total mixture is stirred for another hour at the elevated temperature.

Lipid class composition (g)	I	II	III	*Fatty acid composition of triacylglycerol (wt%)*	
Phosphatidylcholine	2.50				
Monoacylglycerol		2.50			
Triacylglycerol			5.00		
				16:0 palmitate	*10.0*
				18:0 sterarate	*2.8*
				18:1 oleate	*20.6*
				18:2 linoleate	*58.9*
				18:3 linolenate	*6.7*
				minors	*1.0*
Total	2.50	2.50	5.00	*Total*	*100*

Figure 1 shows a microscope photo of the formulation according to Example 9, 4 min after the addition of external water (magnification = 60x). It is apparent from the figure that lipid vesicles, denoted here as Biosomes, are formed from the Biosome Forming Matrix at the interface between aqueous and lipid phase

and that the process seems to occur by means of a spontaneous 'budding' mechanism which takes place immediately after contact with external water.

Figure 2 shows a microscope photo of the formulation according to Example 10, 10 s after the addition of external water (magnification ≈ 60x). As can be seen, worm-like textures are formed in the lipid phase, i.e. the Biosome Forming Matrix, which moves towards the interface between water and lipid. Then at the water-lipid interface, these textures are rapidly transformed by a 'budding' process into spherical lipid vesicles, denoted as Biosomes in this invention.

The *in vitro* release of vitamin B12 from the BFM's in Examples 9 and 10 was tested. The BMF formulations were added to water at 20 °C and then shaken gently for 3 min before measuring the B12 concentration in the aqueous phase. The formulations were allowed to stand for 120 min followed by a repeated analysis. In order to obtain a clear aqueous phase, centrifugation was performed for 30 min at 45,000 rpm before concentration measurements. The results are shown in Table II.

Table II

Time (min)	Release Example 9	Example 10
3	85 %	76 %
120	85 %	84 %

As can be seen, a very rapid and spontaneous release of vitamin B12 was obtained from the two BFM formulations. Also, depending on the lipid composition, different release properties were obtained. Only small changes in the fatty acid composition gave different release properties. The lipid particles according to the experiment given in Table II above, i.e. Biosomes formed from Examples 9 and 10, were subjected to size analysis using a Malvern equipment. The results thus obtained are shown in Table III.

Table III

Example No.	Time (min)	Size
9	3	26 % <1 µm >1 µm 66 % <2 µm
	120	41 % <1 µm >1 µm 46 % <2 µm
10	3	0 % <1 µm >1 µm 96 % <10 µm
	120	44 % <1 µm >1 µm 52 % <2 µm

Initially, smaller Biosomes are spontaneously formed for Example 9 compared to Example 10 as evident from Table III. Furthermore, for the smaller Biosome forming matrices a more rapid drug release can be seen, cf. Table II. Another interesting phenomenon can be seen in Table III in terms of the time for the formation of smaller Biosomes. A longer lag time for this process was found for Example 10 compared to Example 9 which demonstrated the possibility of controlling this process by means of lipid composition in the BFM's.

EXAMPLE 11

2.50 g phosphatidylcholine from soybean (I) and 7.50 g of a monoglyceride (II) are gently stirred for 6 h at 60 °C. 1.25 g water is added and the stirring continues for another hour at the elevated temperature.

Lipid class composition (g)	I	II
Phosphatidylcholine	2.50	
Monoacylglycerol		7.50
Total	2.50	7.50

EXAMPLE 12

2.50 g phosphatidylcholine from soybean (I) and 7.50 g of a monoglyceride (II) are gently stirred for 6 h at 60 °C. 1.25 g Fragmin® solution (120 mg/g water) is added and the stirring continues for another hour at the elevated temperature.

Lipid class composition (g)	I	II
Phosphatidylcholine	2.50	
Monoacylglycerol		7.50
Total	2.50	7.50

EXAMPLE 13

2.50 g phosphatidylcholine from soybean (I) and 7.50 g of a monoglyceride (II) are gently stirred at 60 °C for 6 h. 0.625 g Fragmin® solution (120 mg/g water) is added and the stirring continues for another hour at the elevated temperature.

Lipid class composition (g)	I	II	*Fatty acid composition of monoacylglycerol (wt%)*	
Phosphatidylcholine	2.50			
Monoacylglycerol		7.50		
			8:0 caprylate	*79.6*
			10:0 caprate	*19.8*
			12:0 laurate	*0.2*
			minors	*0.4*
Total	2.50	7.50	*Total*	*100*

EXAMPLE 14

2.50 g phosphatidylcholine from soybean (I) and 7.50 g of a monoglyceride (II) are gently stirred at 60 °C for 6 h. 1.25 g Fragmin® solution (120 mg/g water) is added and the stirring continues for another hour at the elevated temperature.

Lipid class composition (g)	I	II	*Fatty acid composition of monoacylglycerol (wt%)*	
Phosphatidylcholine	2.50			
Monoacylglycerol		7.50		
			8:0 caprylate	*78.4*
			10:0 caprate	*21.2*
			12:0 laurate	*0.2*
			minors	*0.2*
Total	2.50	7.50	*Total*	*100*

EXAMPLE 15

2.50 g phosphatidylcholine from soybean (I) and 7.50 g of a monoglyceride (II) are gently stirred at 60 °C for 6 h. 0.625 g Fragmin® solution (120 mg/g water) is added and the stirring continues for another hour at the elevated temperature.

Lipid class composition (g)	I	II	*Fatty acid composition of monoacylglycerol (wt%)*	
Phosphatidylcholine	2.50			
Monoacylglycerol		7.50		
			8:0 caprylate	*78.4*
			10:0 caprate	*21.2*
			12:0 laurate	*0.2*
			minors	*0.2*
Total	2.50	7.50	*Total*	*100*

The size distribution of the Biosomes formed in water at 37 °C was determined for Example 15 using a Malvern equipment. The BFM formulation was shaken gently in water for 17 h followed by centrifugation in order to separate the lipid phase from the aqueous phase. The result is shown in Table IV.

Table IV

Size	%
<1 μm	36
>1 μm, <2 μm	60

Example 15 was also administered in a rabbit by subcutaneous injection. Blood samples were collected and the plasma concentration of Fragmin® was analyzed as a function of time. The results are shown in Table V.

Table V

Time (h)	Fragmin® plasma concentration (IU/ml)
0	0
1.0	0
2.0	0
2.5	0.051
3.0	0.100
3.5	0.110
4.0	0.127
4.5	0.130
5.0	0.122
5.5	0.126
6.0	0.133
7.0	0.126

As can be seen in Table V, a constant and extended release of Fragmin® was obtained *in vivo*. It seems as if it is now possible to deliver *in vivo* a highly water-soluble high molecular weight compound at a constant rate by means of the present invention.

Examples 16-23 show various formulations based on the present invention demonstrating the flexibility of said invention. The examples show that it is possible to incorporate both highly complex molecules such as vitamin B12 as well as low molecular weight compound, e.g. buspirone and high molecular weight molecules, e.g. fragmentated heparin (Fragmin®) where each bioactive compound possesses different physicochemical properties.

EXAMPLE 16

150 mg cyanocobalamin (B12) is added to 12.50 g of a monoglyceride (II) and the mixture is gently stirred at 60 °C for 3 h. 12.50 g phosphatidylcholine from soybean (I) is added and the stirring continues for 6 h at 60 °C. 25.00 g of a triglyceride (III) is added and the total mixture is stirred for another hour at the elevated temperature.

Lipid class composition (g)	I	II	III	Fatty acid composition of triacylglycerol (wt%)	
Phosphatidylcholine	12.50				
Monoacylglycerol		12.50			
Triacylglycerol			25.00		
				8:0 caprylate	*58.5*
				10:0 caprate	*40.5*
				12:0 laurate	*0.6*
				minors	*0.4*
Total	12.50	12.50	25.00	*Total*	*100*

EXAMPLE 17

150 mg cyanocobalamin (B12) is added to 12.50 g of a monoglyceride (II) and the mixture is gently stirred at 60 °C for 3 h. 12.50 g phosphatidylcholine from soybean (I) is added and the stirring continues for 6 h at 60 °C. 25.00 g of a triglyceride (III) is added and the total mixture is stirred for another hour at the elevated temperature.

Lipid class composition (g)	I	II	III	Fatty acid composition of triacylglycerol (wt%)	
Phosphatidylcholine	12.50				
Monoacylglycerol		12.50			
Triacylglycerol			25.00		
				16:0 palmitate	*10.0*
				18:0 sterarate	*2.8*
				18:1 oleate	*20.6*
				18:2 linoleate	*58.9*
				18:3 linolenate	*6.7*
				minors	*1.0*
Total	12.50	12.50	25.00	*Total*	*100*

EXAMPLE 18

150 mg cyanocobalamin (B12) is added to 33.30 g of a monoglyceride (II) and the mixture is gently stirred at 60 °C for 3 h. 11.10 g phosphatidylcholine from soybean (I) is added and the stirring continues for 6 h at 60 °C. 5.60 g of water is added and the total mixture is stirred for another hour at the elevated temperature.

Lipid class composition (g)	I	II
Phosphatidylcholine	11.10	
Monoacylglycerol		33.30
Triacylglycerol		
Total	11.10	33.30

EXAMPLE 19

15 mg cyanohydroxycobalamin acetate is added to 1.25 g of a glyceride mixture (II) and gently stirred at 60 °C for 3 h. 1.25 g phosphatidylcholine from soybean (I) is added and the stirring continues for 6 h at 60 °C. 2.50 g of a triglyceride (III) is added and the total mixture is stirred for another hour at the elevated temperature.

Lipid class composition (g)	I	II	III	*Fatty acid composition of triacylglycerol (wt%)*	
Phosphatidylcholine	1.25				
Monoacylglycerol		0.63			
Diacylglycerol		0.63			
Triacylglycerol			2.50		
				8:0 caprylate	*58.5*
				10:0 caprate	*40.5*
				12:0 laurate	*0.6*
				minors	*0.4*
Total	1.25	1.25	2.50	*Total*	*100*

EXAMPLE 20

15 mg cyanohydroxycobalamin acetate is added to 1.25 g of a glyceride mixture (II) and gently stirred at 60 °C for 3 h. 1.25 g phosphatidylcholine from soybean (I) is added and the stirring continues for 6 h at 60 °C. 2.50 g of a triglyceride (III) is added and the total mixture is stirred for another hour at the elevated temperature.

Lipid class composition (g)	I	II	III	*Fatty acid composition of triacylglycerol (wt%)*	
Phosphatidylcholine	1.25				
Monoacylglycerol		0.63			
Diacylglycerol		0.63			
Triacylglycerol			2.50		
				16:0 palmitate	*10.0*
				18:0 sterarate	*2.8*
				18:1 oleate	*20.6*
				18:2 linoleate	*58.9*
				18:3 linolenate	*6.7*
				minors	*1.0*
Total	1.25	1.25	2.50	*Total*	*100*

EXAMPLE 21

2.50 g phosphatidylcholine from soybean (I) and 7.50 g of a monoglyceride (II) are gently stirred for 6 h at 60 °C. 2.0 ml Fragmin® solution (334 mg/g water) is added and the stirring continues for another hour at the elevated temperature.

Lipid class composition (g)	I	II
Phosphatidylcholine	2.50	
Monoacylglycerol		7.50
Total	2.50	7.50

EXAMPLE 22

10 mg buspirone hydrochloride is added to 50 mg of a monoglyceride (II) and the mixture is gently stirred for 1 h at 60 °C. 200 mg of a diglyceride (III) and 250 mg phosphatidylcholine from soybean (I) is added and the stirring continues for 3 h at 60 °C. 500 mg of a triglyceride (IV) is added and the total mixture is stirred for another 10 min at the elevated temperature.

Lipid class composition (mg)	I	II	III	IV	*Fatty acid composition of triacylglycerol (wt%)*	
Phosphatidylcholine	250					
Monoacylglycerol		50				
Diacylglycerol			200			
Triacylglycerol				500		
					8:0 caprylate	*58.5*
					10:0 caprate	*40.5*
					12:0 laurate	*0.6*
					minors	*0.4*
Total	250	50	200	500	*Total*	*100*

EXAMPLE 23

20 mg coenzyme Q10 is added to 200 mg of a diglyceride (II) and 250 mg phosphatidylcholine from soybean (I) and the mixture is gently stirred for 6 h at 60 °C. 500 mg of a triglyceride (III) is added and the total mixture is stirred for another hour at the elevated temperature.

Lipid class composition (mg)	I	II	III	*Fatty acid composition of triacylglycerol (wt%)*	
Phosphatidylcholine	250				
Diacylglycerol		200			
Triacylglycerol			500		
				8:0 caprylate	*58.5*
				10:0 caprate	*40.5*
				12:0 laurate	*0.6*
				minors	*0.4*
Total	250	200	500	*Total*	*100*

CLAIMS

1
Lipid particle forming matrix, characterized by, that from a defined system of at least two lipid components chosen from classes of different polarity, in which at least one of the lipid component is bilayer forming, discrete lipid particles are formed spontaneously when interacting with excess aqueous medium.

2
Lipid particle forming matrix according to Claim 1, characterized by that in the system at least one of the lipid components is amphiphatic and polar and one is nonpolar.

3
Lipid particle forming matrix according to any of Claims 1 or 2, characterized by that the nonpolar lipid is chosen from the class of mono-, di- and triglycerides or a mixture thereof.

4
Lipid particle forming matrix according to any of Claims 1-3, characterized by that the nonpolar lipid contains a triglyceride with essentially a mixture of 8:0 caprylate and 10:0 caprate.

5
Lipid particle forming matrix according to any of Claims 1-3, characterized by that the nonpolar lipid contains a triglyceride with essentially a mixture of 18:2 linoleate, 18:1 oleate and 16:0 palmitate.

6
Lipid particle forming matrix according to any of Claims 1-3, characterized by that the nonpolar lipid contains a monoglyceride with essentially a mixture of 8:0 caprylate and 10:0 caprate.

7
Lipid particle forming matrix according to Claim 2, characterized by that the amounts of the amphiphatic and polar lipid components are in the range of 5-80 % (w/w) of the lipid system, preferably in the range of 10-60 % (w/w).

8
Lipid particle forming matrix according to Claim 7, characterized by that the amphiphatic and polar lipid components are bilayer forming and in an amount of 25-50 % (w/w) of the lipid system.

9
Lipid particle forming matrix according to any of Claims 2 and 7-8, characterized by that the amphiphatic and polar lipid components are chosen from phosphatidylcholine, and other phospholipids.

10
Lipid particle forming matrix according to any of Claims 2 and 8-9, characterized by that the amphiphatic and polar lipid components is phosphatidylcholine in an amount of 50 % (w/w).

11
Lipid particle forming matrix according to any of the Claims 1-10, characterized by that the lipid particle forming matrix contains bioactive materials.

12
Lipid particle forming matrix according to Claim 11, characterized by that the bioactive material is chosen from the groups of drugs, herbicides, pesticides, fertilizers, food and cosmetic ingredients or additives.

13
Lipid particle forming matrix according to Claims 11 and 12, characterized by that the amount of bioactive material is below 70% (w/w) of the matrix, preferably below 50% (w/w).

14
Lipid particle forming matrix according to Claims 1-10, characterized by that discrete particles are formed spontaneously from the matrix without any chemical or physical treatment or initiation.

15

Process for the production of a lipid particle forming matrix according to any of the Claims 11-13, characterized by that the amphiphatic and polar or the nonpolar lipid is mixed with the bioactive material *per se* or in solution.

16

Process according to Claim 15, characterized by a first admixing of nonpolar lipid or lipids with the bioactive material and thereafter admixing with the amphiphatic and polar lipid or lipids.

17

Use of the lipid particle forming matrix according to any of Claims 1-10, as a carrier system for bioactive materials.

18

Pharmaceutical composition such as oral, rectal, nasal, vaginal, ocular or parenteral vehicles, creams, ointments, capsules and tablets containing the said lipid particle forming matrix according to any of Claims 11-13 and a carrier.

19

Use of the lipid particle forming matrix according to any of Claims 11-13, for the manufacturing of a pharmaceutical composition for enteral, parenteral, nasal, intravaginal, ocular adminstration or adminstration locally on skin, wounds or mucous membranes.

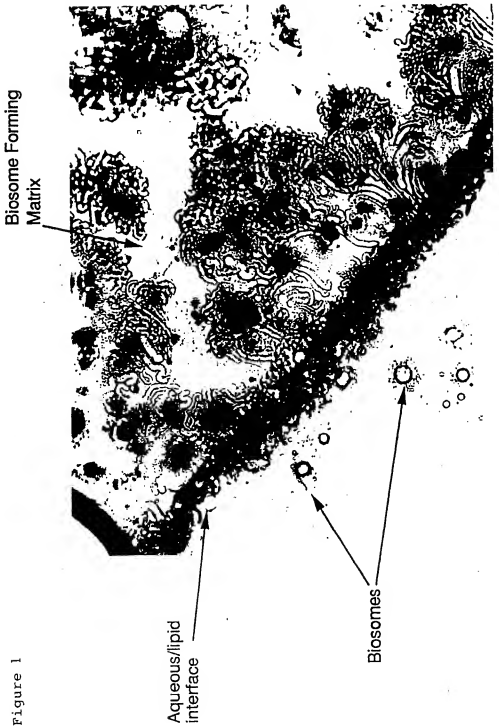

Figure 1

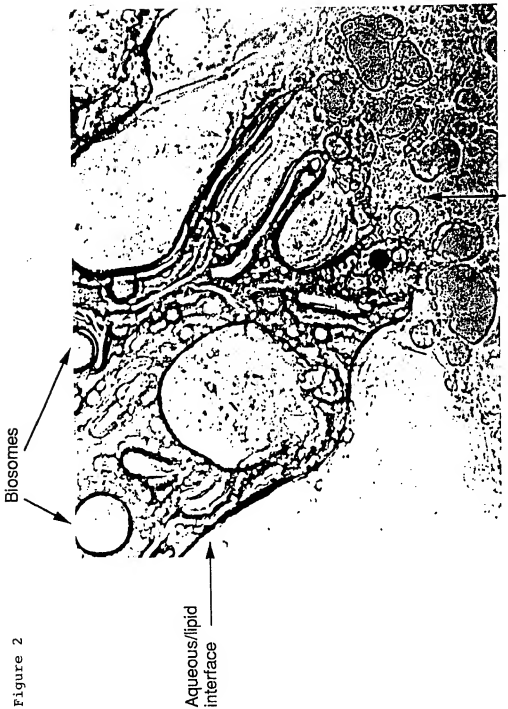

Figure 2

INTERNATIONAL SEARCH REPORT

International Application No PCT/SE 91/00639

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: A 61 K 9/127, 47/44, B 01 J 13/02

II. FIELDS SEARCHED

Minimum Documentation Searched[7]	
Classification System	Classification Symbols
IPC5	A 61 K; B 01 J

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in Fields Searched[8]

SE,DK,FI,NO classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	EP, A, 0158441 (PHARES PHARMACEUTICAL RESEARCH N.V.) 16 October 1985, see page 5, line 1 - page 6, line 16; page 8, line 5 - page 9, line 18; page 12, line 18 - line 34 --	1-3,9, 11-19
X	EP, A, 0260241 (AKTIEBOLAGET DRACO) 16 March 1988, see page 3, line 20 - line 63 --	1-2,9, 11-19
X	WO, A1, 8707502 (PHARES PHARMACEUTICAL RESEARCH N.V.) 17 December 1987, see pages 1-3, example 3 and claims --	1-3,9, 11-19
A	EP, A, 0084169 (AUSONIA FARMACEUTICI SRI) 27 July 1983, see the whole document --	1-19

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
14th January 1992	1992 -01- 15
International Searching Authority	Signature of Authorized Officer
SWEDISH PATENT OFFICE	Anneli Jönsson

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	Journal of Pharmaceutical Sciences, 75(1986-04):4, N.I. Payne et al: "Characterization of proliposomes" see the entire article --	1-19
A	US, A, 04610868 (MW FOUNTAIN ET AL) 9 September 1986, see the whole document --	1-19

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.PCT/SE 91/00639

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the Swedish Patent Office EDP file on 30/11/91
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0158441	85-10-16	JP-A- 61044808	86-03-04
		US-A- 5004611	91-04-02
EP-A- 0260241	88-03-16	AU-B- 603139	90-11-08
		AU-D- 7913387	88-04-07
		CA-A- 1256798	89-07-04
		EP-A- 0282537	88-09-21
		JP-T- 1500668	89-03-09
		WO-A- 88/01862	88-03-24
		ZA-A- 8706641	88-03-14
WO-A1- 8707502	87-12-17	EP-A- 0309464	89-04-05
EP-A- 0084169	83-07-27	NONE	
US-A- 04610868	86-09-09	NONE	

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Published
With international search report.
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(54) Title: IMPROVED PERSONAL CARE FORMULATIONS

(57) Abstract

Personal care and hygiene formulations for topical application to mucosal surfaces. These formulations include an amphiphilic lipid carrier in the form of a colloidal composition which can include a micellar aggregate or mixed micelles dispersed in a continuous aqueous phase, or an emulsion of lipid droplets suspended in a continuous aqueous phase, and an active agent which is an anti-microbial agent. The lipid carrier has high adhesiveness to mucous membranes such as the soft tissues of the oral cavity. The lipid carrier also has a high load capacity for the active agent to be carried to these tissues. These formulations have the desirable properties of carrying a large amount of active agent for controlled and prolonged release thereof at the desired site, such as mucous membrane surfaces and surrounding tissue. Accordingly, the present invention provides a formulation for oral or topical application including an anti-microbial agent and a lipid. The agent is held by the carrier through a hydrophobic interaction and is released from the carrier in a controlled manner over a prolonged period of time. The lipid is also characterized by having a high adhesive capability towards mucous membrane surfaces. The lipid and the agent are preferably present in a ratio in a range of from about 1:10 to about 10:1, more preferably from about 1:5 to about 5:1, and most preferably from about 1:3 to about 3:1 in the formulation.

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Improved Personal Care Formulations

Field and Background of the Invention

The present invention relates to new improved formulations for application to a mucosal tissue, and to methods of preparation of these formulations. These formulations are useful for oral administration, such as mouth wash or oral rinse formulations. More specifically, the present invention concerns improved formulations including a lipid carrier and biologically active agent dispersed in a continuous aqueous phase. The lipid carrier is characterized by having high adhesive capabilities towards mucous membranes such as those of the gums, tongue and palate. The lipid carrier also has a high load capacity for the biologically active agent. As such, the lipid carrier can specifically target a relatively large amount of the agent to these mucous membranes to ensure a controlled and sustained release of the agent at the mucous surface.

In the field of personal care and hygiene, many different formulations have been designed and employed commercially in a wide variety of "over-the-counter" medications and products for a number of purposes including oral hygiene and skin care. Many of these medications and products contain both a biologically active agent such, as for example, an anti-microbial agent, and an inert vehicle. The particular choice of vehicle depends upon the desired properties of the formulation.

However, the currently available formulations for personal care and hygiene products suffer from a number of drawbacks, including lack of suitability of the carrier for its intended use. Most of these known formulations suffer from an inability to carry a large amount of the active agent and to ensure a controlled and prolonged release thereof at the desired site. This inability is particularly undesirable, since usually any biologically active agent must remain at the desired site for a prolonged period in order to be effective.

Recently, liposome-based delivery systems have been developed in which the active agent is encapsulated within a multilamellar lipid vesicle or liposome, and is then released in a controlled fashion from the liposome. For example, U.S. Patent No. 4,588,578 discloses lipid vesicles in which the active ingredient is encapsulated, rather than being complexed with a lipid. However, such liposomes suffer from the drawback of having a limited load capacity for the active agent.

Furthermore, many of these liposomes and related lipid particles are not suitable for long term storage, particularly at ambient temperatures. An example of a liposome-based delivery system has been disclosed in U.S. Patent No. 4,767,615, in which specific modifications to the lipid structure enable specific targeting of the liposome to specific tissues, such as the enamel of the teeth. Conversely, the very specificity of such carriers limits them to tissues covered by an enamel layer. Furthermore, the maximum capacity for the active agent is only about 20% of the liposome volume of the disclosed prior art carrier.

As another example, U.S. Patent No. 5,415,867 discloses lipid particles with a relatively high ratio of agent to lipid. However, this reference does not teach or disclose the use of such particles for administration to a mucosal tissue or mucous membrane. Instead, the reference primarily teaches parenteral administration. Similarly, PCT Application No. WO 92/03121 discloses only colloidal particles for oral administration or for administration on the intact skin. Thus, the prior art does not teach the use of high ratio lipid particles for administration to a mucous membrane or mucosal surface.

Furthermore, the known non-liposome, hydrophilic, water soluble formulations also suffer from a very short retention time at the tissue to which they are applied, because they are readily washed away or degraded.

In view of the above drawbacks of the prior art carriers, there has been a long-felt need to provide formulations for personal care and hygiene which are multi-purpose and can be applied to a mucosal tissues. Such carriers must have high adhesion capability to ensure contact for a prolonged time, and must be able

to carry a high amount of active agent to the site of adhesion for a controlled and prolonged release to the desired tissue.

Other aims and aspects of the present invention will be apparent from the following description of the present invention.

Summary of the Invention

The present invention concerns new personal care and hygiene formulations for topical application to mucosal surfaces. These formulations include an amphiphilic lipid carrier in the form of a colloidal composition which can include a micellar aggregate or mixed micelles dispersed in a continuous aqueous phase, or an emulsion of lipid droplets suspended in a continuous aqueous phase, and an active agent which is an anti-microbial agent. The lipid carrier has high adhesiveness to mucous membranes such as the soft tissues of the oral cavity. The lipid carrier also has a high load capacity for the active agent to be carried to these tissues.

These formulations have the desirable properties of carrying a large amount of active agent for controlled and prolonged release thereof at the desired site, such as mucous membrane surfaces and surrounding tissue. Accordingly, the present invention provides a formulation for oral or topical application including an anti-microbial agent and a lipid. The agent is held by the carrier through a hydrophobic interaction and is released from the carrier in a controlled manner over a prolonged period of time. The lipid is also characterized by having a high adhesive capability towards mucous membrane surfaces. The lipid and the agent are preferably present in a ratio in a range of from about 1:10 to about 10:1, more preferably from about 1:5 to about 5:1, and most preferably from about 1:3 to about 3:1 in the formulation.

According to the present invention, there is provided a formulation for topical application to a tissue selected from the group consisting of nasal, ophthalmic, oral cavity, vaginal and rectal, the formulation including: (a) a

biologically active agent selected from the group consisting of antibiotic, antiviral agent, antifungal agent, disinfectant, nutrient, anti-inflammatory agent, local anesthetic and essential oil; and (b) a lipid carrier, the lipid carrier including at least one lipid selected from the group of amphiphilic phospholipids consisting of egg yolk lecithin, phosphatidic acid, alkylphosphates, phosphatidylglycerol, Soya lecithin and phosphatidyl choline, the lipid being characterized as a colloidal dispersion or as an emulsion of lipid droplets in suspension in an aqueous medium, and the lipid and the active agent being present in a ratio of from about 10:1 to about 1:10, such that the agent is carried by the lipid carrier and the agent is released from the carrier in a controlled manner and over a prolonged period of time.

Hereinafter, the term "topical" refers to direct application to an external surface or to a cavity of tissues of the body. The term "ophthalmic" refers to the tissue at the external surface of the eye or the external surfaces of surrounding tissues. The term "oral cavity" includes the surface of the mouth, lips, tongue and gums.

Preferably, the antibiotic is selected from the group consisting of erythromycin, tetracycline, and chloramphenicol. Preferably, the antiviral agent is selected from the group consisting of azothymidin, acyclovir, dideoxyuridine and amantadine. Preferably, the antifungal agent is selected from the group consisting of ketoconazole, fluconazole, miconazole, tolnaftate, amphotericin and nystatin. Preferably, the disinfectant is selected from the group consisting of chlorhexidine and salts thereof, triclosan, cetrimide and cetylpyridinium chloride. Preferably, the nutrient is selected from the group consisting of vitamin A, vitamin E, vitamin D, vitamin K, ascorbyl palmitate, coenzyme Q-10, coenzyme Q-50, lipoic, biotin and carnitine. Preferably, the anti-inflammatory agent is selected from the group consisting of non-steroidal and steroidal. More preferably, the non-steroidal anti-inflammatory agent is selected from the group consisting of indomethacin, ketoprofen, diclofenol and acetylsalicylic acid. Alternatively and more preferably,

the steroidal anti-inflammatory agent is selected from the group consisting of dexamethazone, prednisolone and fluoromethzolone acetonide. Preferably, the local anesthetic is selected from the group consisting of lidocaine, trimecaine and benzocaine. Preferably, the essential oil is selected from the group consisting of menthol, vanillin, peppermint oil, clove oil, eucalyptus oil and lavender oil.

Preferably, the agent is further characterized by having activity in the oral cavity for treatment of at least one condition selected from the group consisting of gum disease, caries, dry mouth, malodorous breath, and microbial infection. More preferably, the microbial infection includes an infection selected from the group consisting of bacterial, viral and fungal.

Alternatively and preferably, the agent is further characterized by having activity on a tissue selected from the group consisting of vaginal and rectal, the activity being suitable for treatment of at least one condition selected from the group consisting of inflammation, irritation, dryness and microbial infection.

According to other preferred embodiments of the present invention, the lipid and the agent are present in a ratio of from about 5:1 to about 1:5. More preferably, the lipid and the agent are present in a ratio of from about 3:1 to about 1:3.

According to a preferred embodiment of the present invention, the formulation preferably further includes a stabilizer, the stabilizer including at least one surfactant selected from the group consisting of non-ionic, anionic, cationic and amphiphilic. Preferably, the stabilizer is a non-ionic surfactant selected from the group consisting of polyethylene glycol derivatives and glycerol derivatives. More preferably, the polyethylene glycol derivative is selected from the group consisting of Tweens, tritons, tyloxapol, pluronics, Brijes, Spans, poloxamers and emulphors. Also more preferably, the glycerol derivative is selected from the group consisting of polyglycerines and polyalkylglycerides.

Alternatively and preferably, the stabilizer is an anionic surfactant selected from the group consisting of alkyl and aryl sulphonates and phosphates. Also

alternatively and preferably, the stabilizer is a cationic surfactant selected from the group consisting of cethyl pyridinium chloride or bromide, and cethyl trimethylammonium bromide. Alternatively and preferably, the stabilizer is an amphiphilic surfactant selected from the group consisting of alkyl betaine derivatives, cocoamphodiacetale derivativcs, lauroamphoacetates and phosphatidylglycerol.

According to another preferred embodiment of the present invention, the formulation preferably also includes at least one lipid additive selected from the group consisting of triglycerides, alkyl esters, cholesterol, triolein, Soya oil, medium chain glycerides, isopropylmyristate and cholesterol esters.

According to still another preferred embodiment of the present invention, the formulation further includes at least one additive selected from the group consisting of flavors, aroma modifiers, sweeteners, colors, and antioxidants.

According to yet another preferred embodiment of the present invention, the formulation includes a lipid in a form selected from the group consisting of micelles, mixed micelles and micellar aggregates, the lipid having a particle size of from about 10 to about 300 nm. Alternatively and preferably, the lipid is in a form selected from the group consisting of an emulsion and a suspension, the lipid having lipid particles of size in the range of from about 50 to about 300 nm.

According to another embodiment of the present invention, there is provided a method for the preparation of a formulation for topical application to a tissue selected from the group consisting of ophthalmic, oral cavity, vaginal and rectal, the method including the steps of: (a) dissolving the lipid and the agent in a water-miscible solvent to form a solution; and (b) adding water to the solution in an amount sufficient to dilute the water-miscible solvent to form a diluted solution. Preferably, the water-miscible solvent is selected from the group consisting of ethyl alcohol, propylene glycol and polyethylene glycol (PEG). Also preferably, the method further includes the step of: (c) passing the diluted solution

through a microporous membrane having a pore size selected from the group consisting of 0.05 micron, 0.1 micron, 0.2 micron, 0.45 micron and 0.8 micron.

According to still another embodiment of the present invention, there is provided a method for the preparation of a formulation for topical application to a tissue selected from the group consisting of ophthalmic, oral cavity, vaginal and rectal, the method including the steps of: (a) mixing the lipid and the agent to form a substantially clear solution; (b) mixing the clear solution with water to form a diluted suspension; and (c) sizing the diluted suspension to form a homogenized suspension. Preferably, the method further includes the step of: (d) filtering the homogenized suspension with a microfilter.

According to yet another embodiment of the present invention, there is provided a method of administering a formulation to a mucosal tissue selected from the group consisting of nasal, ophthalmic, oral cavity, vaginal and rectal, comprising the steps of: (a) providing the formulation, the formulation featuring: (i) a biologically active agent selected from the group consisting of antibiotic, antiviral agent, antifungal agent, disinfectant, nutrient, anti-inflammatory agent, local anesthetic and essential oil; and (ii) a lipid carrier, the lipid carrier including at least one lipid selected from the group of amphiphilic phospholipids consisting of yolk lecithin, Soya lecithin, phosphatidylglycerol and analogs thereof, the lipid being characterized as a colloidal micellar dispersion or as an emulsion of lipid droplets dispersed in an aqueous medium, and the lipid and the agent being present in a ratio of from about 10:1 to about 1:10, such that the agent is carried by the lipid of the lipid carrier and the agent is released from the lipid in a controlled manner and over a prolonged period of time, and such that the lipid carrier has a property of high adhesion to the mucosal tissue; and (b) administering the formulation to the mucosal tissue. Preferably, the mucosal tissue is the oral cavity and the formulation is administered as a mouthwash.

Brief Description of Drawing

The invention is herein described, by way of example only, with reference to the accompanying drawing, wherein:

FIG. 1 is a graph of the effect of the formulation of the present invention.

Detailed Description of the Invention

The present invention concerns new improved formulations for local oral and other topical mucosal applications which contain a biologically active agent. These formulations are therefore particularly useful for the purposes of oral hygiene and for the purposes of antiseptic treatment of the mucosal surface.

More specifically, the present invention concerns formulations containing micelles or self-emulsifying compositions having a biologically active agent, which have a high adhesive capacity for mucous membranes such as those on the outer surfaces of the gums. These colloidal compositions also have a large capacity for the anti-microbial agent. The lipid components of the micelles or emulsion interact with the agent through non-covalent hydrophobic attraction.

The formulations of the present invention are particularly well suited for administering the anti-microbial agent in effective amounts to mucosal surfaces where the agent is released by a slow-release process over a prolonged period. These formulations are useful as mouth wash formulations for oral hygiene. After contacting the oral cavity, the carrier with the anti-microbial agent will first adhere to the mucosal surface of the gums, and the agent will then be released to the surrounding teeth and oral cavity in a substantially continuous manner over a prolonged time. Indeed, effective amounts of the anti-microbial agent could potentially be present for as long as 24 hours, requiring oral application of the formulation only about once a day. Such oral formulations are therefore effective for maintaining general oral hygiene and specifically to combat tooth decay, gum disease and malodorous breath.

These desirable characteristics of the formulations of the present invention were achieved by preparing a formulation in which the ratio of lipid to

biologically active agent was reduced from prior art formulations, which relied heavily on employing large amounts of lipid to carry effective amounts of the active ingredient. In addition, the lipid carrier is needed to target the active agent and cause it to adhere to the desired tissue, and then to release this agent in a controlled manner. Prolonged, controlled release of the biologically active agent is especially important because such release of such a biologically active agent provides for optimal biological effects and, at the same time, also reduces the absolute amount of the agent necessary for the desired effect. Reduction of the total amount of the active ingredient could decrease adverse side effects, which are usually dose dependent.

Although the Examples are drawn to specific active ingredients, namely chlorhexidine and triclosan, these are for illustrative purposes only and are not meant to be limiting. It is anticipated that formulations according to the present invention would also be effective for a number of other active ingredients, which can be divided into the following groups: antibiotic, antiviral agent, antifungal agent, disinfectant, nutrient, anti-inflammatory agent, local anesthetic and essential oil.

Examples of each of these groups are listed herein, it being understood that these examples are for illustrative purposes only and are not meant to be limiting in any way. Preferably, the antibiotic is selected from the group consisting of erythromycin, tetracycline, and chloramphenicol. Preferably, the antiviral agent is selected from the group consisting of azothymidin, acyclovir, dideoxyuridine and amantadine. Preferably, the antifungal agent is selected from the group consisting of ketoconazole, fluconazole, miconazole, tolnaftate, amphotericin and nystatin. Preferably, the disinfectant is selected from the group consisting of chlorhexidine and salts thereof, triclosan, cetrimide and cetylpyridinium chloride. Preferably, the nutrient is selected from the group consisting of vitamin A, vitamin E, vitamin D, vitamin K, ascorbyl palmitate, coenzyme Q-10, coenzyme Q-50, lipoic, biotin and carnitine. Preferably, the anti-inflammatory agent is selected from the group

consisting of non-steroidal and steroidal. More preferably, the non-steroidal anti-inflammatory agent is selected from the group consisting of indomethacin, ketoprofen, diclofenol and acetylsalicylic acid. Alternatively and more preferably, the steroidal anti-inflammatory agent is selected from the group consisting of dexamethazone, prednisolone and fluoromethzolone acetonide. Preferably, the local anesthetic is selected from the group consisting of lidocaine, trimecaine and benzocaine. Preferably, the essential oil is selected from the group consisting of menthol, vanillin, peppermint oil, clove oil, eucalyptus oil and lavender oil.

The formulations of the present invention preferably have a ratio of biologically active agent to lipid of from about 1:10 to about 10:1, more preferably of from about 1: 5 to about 5:1 and most preferably from about 1:3 to about 3:1. The high mucosal adhesive property of this delivery system is determined by the lipid molecules at the surface of the particles. Optionally and preferably, there is also included stabilizing agents, in the form of anionic and non-ionic surfactants, which serve to stabilize the lipid-biologically active agent complex at the desired ratio.

Preferred formulations of the present invention include those having chlorhexidine or triclosan as the biologically active agent, which in their case, serve as anti-microbial agents. These preferred formulations are intended primarily for personal hygiene products including mouth wash-formulations and chewing gum, and cosmetic products including various formulations and liquid soaps.

In the preferred formulations of the invention, the lipid component is in the form of micelles, mixed micelles or micellar aggregates, or in the form of emulsions (lipid colloids with an inner lipid phase or fatty phase) which provide for only an external association between the lipid and the biologically active agent, as opposed to liposomes which have a structure consisting of an inner hydrophilic core which contains the biologically active agent. The interaction between the biologically-active agent and the lipid is via hydrophobic interactions.

Such interactions therefore enable the lipid to associate with a large amount of biologically active agent over the entire surface of the lipid micelle or emulsion to provide a high load capacity for the biologically active agent of at least about 10% and up to about 90%, more preferably at least 25% and up to about 80%, of the weight of the lipid phase. The lipid itself causes the strong adhesion of the dispersed formulation to the mucous membranes of the oral cavity and to other mucosal tissues. Without wishing to be bound by a particular mechanism, presumably the adhesive property of the formulation is due to the amiphiphilic characteristics of the lipid.

For example, in mouth wash formulations in accordance with the present invention, the lipid-biologically active agent ratio is of such a nature that a single use of the mouth wash solution will provide gum and teeth protection, and prevent the occurrence of malodorous breath for approximately a full day (24 hours), even if the user eats and drinks during this period. In addition to the above essential components of the formulations, stabilizers (preferably anionic and non-ionic surfactants) are also preferably employed to stabilize the interaction between the lipid and biologically active agent, which enables maximum loading of the lipid micelles or emulsions with the biologically active agent, as well as stabilization of the release of the biologically active agent at the desired site.

The lipid components of the formulations of the present invention, whether in the form of micelles, mixed micelles or micellar aggregates, or emulsions, are organized into aggregates of particular size distribution of from about 10 nm to about 300 nm, this providing the above noted high adhesion capability of the lipid aggregates to mucosal membranes and enabling both a high load capability of the biologically active agent onto the lipid aggregates and a prolonged release period of the biologically agents from the lipid aggregates. The structure of the lipid aggregates includes hydrophobic hydrocarbon chains of the lipid molecules at the core and polar groups of the lipid molecules at the surface, thereby enabling these lipid aggregates to be formulated into the preferred

aqueous formulations of the present invention. Also, the structure provides for effective interaction with the preferred biologically active agents of the present invention. The improved properties of this formulation over previously known formulations are achieved by forming the suspension with lipid or lipophilic particles which are highly adhesive to mucosal membranes, and which permit prolonged and controlled release of the biologically active agent from the lipid particles at the mucosal surface.

More preferably, the formulation is an aqueous lipid colloidal formulation for application to a mucosal surface, in a particular, an oral mucosal membrane surface as found on the gums. This formulation includes a pharmaceutically acceptable anti-microbial agent that is distributed between an aqueous phase and suspended small water-insoluble particles in a colloidal dispersion.

The preparation of the formulations of the present invention includes well known standard chemical techniques well known to those of skill in the art as set forth in a large number of chemical texts readily available to skilled artisans.

As the formulations of the present invention are preferably non-medical formulations intended for over-the-counter distribution to the public, the ingredients of the formulations of the present invention have preferably been approved for this purpose by the relevant health authorities. Examples of the various components of the formulations of the present invention are the following.

First, lipids which have high adhesive capability to mucosal membranes include the various amphiphilic lipids such as the phospholipids, for example, egg yolk lecithin, Soya lecithin and phosphatidylcholine. Preferably such lipids will be used at a concentration of from about 0.1 to about 5% in the formulations. At this concentration an optimally bioadhesive particle will be obtained.

Suitable biologically active agents include agents which can be used to treat an existing condition of the skin, or of the rectal, vaginal or oral cavities, or to prevent such a condition from arising as a prophylactic measure. For example, preferably the agent is further characterized by having activity in the oral cavity

for treatment of at least one condition selected from the group consisting of gum disease, caries, dry mouth, malodorous breath, and microbial infection. Hereinafter, any agent which is active against a microbe is referred to as an "anti-microbial agent". Hereinafter, the term "microbial infection" includes bacterial, viral and fungal infections.

Alternatively and preferably, the biologically active agent is suitable for treatment of at least one condition selected from the group consisting of inflammation, irritation, dryness and microbial infection on a tissue selected from the group consisting of vaginal and rectal.

If an anti-microbial agent is to be used, suitable anti-microbial agents include the known, approved, multi-purpose agents included with various liquid antiseptics and disinfectants, such as triclosan and chlorhexidine. Preferably, triclosan is used in a concentration of from about 0.01% to about 2.0% in the final formulations, and chlorhexidine is used in a concentration of from about 0.001% to about 2% in the final formulations, when these formulations are ready for administration.

It should be noted that the two essential ingredients are the lipid and the biologically active agent. However, additional ingredients may be optionally added to the formulation to achieve certain desired characteristics. According to a preferred embodiment of the present invention, a suitable stabilizer is preferably included. Stabilizers of the lipid and anti-microbial agent complex are generally surfactants which stabilize the interaction between the lipids and the anti-microbial agent in the formulations. These stabilizers thus serve to increase the load capability of the lipids, control the release of the active agent from the lipids over a long period, and also improve the reological properties of the formulations (viscosity of the formulations). The surfactants may be of a number of types, including non-ionic surfactants such as polyethylene glycol derivatives and glycerol derivatives. The polyethylene glycol derivatives can be, for example, polyoxyethylated including the various Tweens, tritons, tyloxapol, pluronics,

Brijes, Spans, poloxamers and emulphors. The glycerol derivatives can be for example, polyglycerines or polyalkylglycerides. When such non-ionic surfactants are used in the formulations, the concentration is preferably in the range of from about 0 to about 5%. These non-ionic surfactants are particularly useful for improving the reological properties (viscosity) and stability of the formulations.

Suitable anionic surfactants include the various alkyl and aryl sulphonates and phosphates such as, for example, the various stearates (e.g. sodium lauryl sulfate), oleates or palmitates. When those are used in the formulations, their concentration is preferably in the range of from about 0 to about 0.5%. These anionic surfactants are particularly useful for improving the loading of the anti-microbial agent onto the lipid particles in the formulations. Furthermore, in this colloidal composition, the addition of anionic surfactants such as sodium stearate does not detract from the activity of chlorhexidine. Such a finding is contrary to the teachings of the prior art, in which the addition of anionic surfactants to prior art formulations of chlorhexidine resulted in a loss of activity.

Suitable cationic surfactants include cethyl pyridinium chloride or bromide, or cethyl trimethylammonium bromide, preferably at a concentration in the range of from about 0 to about 2%. These cationic surfactants are particularly useful for improving the antiseptic activities of triclosan or chlorhexidine in the formulations.

Suitable amphiphilic surfactants include the various alkyl betaines, cocoamphodiacetales or lauroamphoacetates, as well as phosphatidylglycerol. Preferably, the concentration is in the range of from about 0 to about 2%.

It should be noted that a mixture of two or more of the above surfactants may be used in the formulations of the present invention, which is preferred, each surfactant improving the properties of the formulation in its own specific way.

An additional optional ingredient is an additional lipid moiety. Suitable lipid moities include the various triglycerides, alkyl esters and cholesterol, such as, for example, triolein, Soya oil, miglyol; isopropylmyristate; and cholesterol esters.

Preferably, the concentration is in the range of from about 0 to about 30%. These additives are particularly useful in the preparation of emulsions and serve to increase the total amount of the active agent carried by the lipid particles.

Another optional but preferred ingredient is a flavor or aroma modifier. Suitable flavor or aroma modifiers include the various approved natural or synthetic flavoring or aroma substances such as, for example, vanillin, menthol, peppermint oil, thyme oil and the like. When used in the formulations, their amount is that quantity specified by the manufacturer or as acceptable in the art. These additives are particularly useful in those formulations of the invention intended for use as oral formulations such as a mouth wash, oral rinse or the like.

Still another optional ingredient is a sweetener. Suitable sweeteners include the various food grade sweeteners such as aspartame, sorbitol, glycerol, mannitol, saccharine, cyclamates and the like. When used their amount is usually specified by the manufacturer or as acceptable in the art. These additives are particularly useful in the oral formulations of the invention.

Other optional ingredients include a coloring agent. Suitable coloring agents include the various food grade colors, such as, for example, beta-carotene, methylene blue and the like. When used, their amount is that specified by the manufacturer or as acceptable in the art. These additives are particularly useful in oral formulations of the invention.

Finally, another optional ingredient is an antioxidant. Suitable antioxidants and other stabilizers include the various tocopherols, ascorbates, and helates such as EDTA. Preferably the concentration is in the range of from about 0.001 to about 0.2%. These additives are particularly useful to improve the stability of the formulations during storage and to prolong shelf-life.

As mentioned above, the various lipids, biologically active agents and additives of the formulations of the invention are known and widely available from a member of commercial suppliers. Methods of preparation are also known. However, in accordance with the present invention there is also provided specific

preferred methods to prepare these formulations. These methods include processes for the preparation of bioadhesive colloidal antiseptic compositions, which are particularly useful for preparing stable oral rinse formulations. One example of such a method starts with the dissolution of the biologically active agent, the lipid, and any additional ingredients such as stabilizers and antioxidants, in a minimal amount of a water-miscible solvent, such as ethyl alcohol. Next, the ingredients are mixed with an appropriate amount of water.

This will provide the desired suspension of liquid particles as a colloidal dispersion in the water phase with the antiseptic distributed between the water phase and the suspended lipid particles. If necessary, the suspension can be filtered through a microporous membrane, preferably with a pore size of from about 0.1 to about 0.45 microns, to improve the particle size distribution and suspension stability. Alternatively, the raw, original suspension can be treated in any suitable known high pressure homogenizer to reduce particle size as is well known in the art. Following this homogenization step, the suspension can be optionally filtered through a microporous membrane as noted above.

In formulations containing lipid emulsions in which lipid additives are also included, the same procedure as above may be employed to improve and control particle size. In addition, in such formulations a self-dispersion process may be used followed by homogenization of the coarse dispersion to yield the desired submicron colloidal formulation having improved stability.

It should be noted, however, that the optimal method for preparing each formulation of the invention is dependent upon the choice of the ingredients for each formulation and the steps of the method will be chosen accordingly to the properties of the various components, their behavior in solution or suspension and their concentration. Such modifications of the method are readily apparent to those of ordinary skill in the art.

The present invention will now be described in more detail with the following non-limiting Examples.

Example 1:Chlorhexidine in colloidal composition without additional surfactants

315 mg (~ 0.4 mmol) of purified egg lecithin (E-80) and 115 mg (~ 0.18 mmol) of chlorhexidine diacetate were dissolved together in 5 ml of ethyl alcohol while stirring to obtain a stock solution. The stock solution was diluted with distilled water during intensive stirring until 45 ml of water was added, such that the final concentration of ethyl alcohol was 10% to obtain a suspension. The suspension was further filtered through a microporous membrane filter of pore size 0.45 micron to form a stable suspension of uniformly sized particles. The mean particle size was 285 $\pm$ 65 nm. About 50% of chlorhexidine was bound to lipid particles, as determined by centrifugal ultrafiltration. The absence of a liposomal fraction in the suspension was determined by NMR.

The high density of lecithin molecules on the particle surface should increase the opportunity for the amphiphilic phosphatidylcholine molecules to interact with polar groups of mucosal tissues. Antimicrobial activity of chlorhexidine was not altered (data not shown).

Example 2. Chlorhexidine colloidal formulation with anionic surfactant

580 mg (0.8 mmol) of lecithin (E-80), 250 mg of chlorhexidine diacetate (0.4 mmol) and 235 mg (0.8 mmol) of sodium lauryl sulfate (SLS) were dissolved in 4 ml of ethyl alcohol. After dilution with 96 ml of distilled water, the resultant suspension was filtered sequentially through membrane filters having a pore size of first 0.45 micron and then 0.22 micron. A stable suspension with particles of a size less than 200 nm was obtained. More than 70% of chlorhexidine was associated with the lipid phase. The antimicrobial activity of chlorhexidine in the prepared colloidal formulation was tested "in vitro" by diffusion in agar plates and by serial dilution. The activity was in the same range as the activity of chlorhexidine in solution.

Example 3. Chlorhexidine colloidal formulation with additional non-ionic surfactant

A formulation was prepared as in Example 2 with Lecithin E-80, but instead of sodium lauryl sulfate (SLS), 100 mg of polyoxyethylene sorbitan monooleate (Tween-80) was added to the alcohol solution. After dilution and filtration through a 0.22 micron membrane filter, a fine suspension was obtained, with a mean particle size of about 60 nm. About 50% of the total chlorhexidine was associated with lipid particles.

Example 4. Chlorhexidine colloidal formulation with additional anionic and non-ionic surfactants, treated with a high pressure homogenizer.

The formulation was prepared by dissolving 500 mg (0.68 mmol) of lecithin E-80, 125 mg (0.2 mmol) of chlorhexidine diacetate, 120 mg (0.4 mmol) of SLS and 120 mg of Tween-80 in a mixture of 2 ml absolute ethyl alcohol and 3 ml 2-propanediol (propylene glycol) to form a stock solution. The stock solution was diluted with 95 ml of distilled water and 2 g of glycerol was added to form a suspension. The suspension was treated with a high pressure homogenizer (EmulsiFlex® C-5 ,"Avestin", Ottawa, Canada), 6 cycles at 12000-15000 psi. The final particle size was about 50 nm with 85% of the drug bound to particles.

Example 5. Chlorhexidine mouthwash colloidal formulation

A mouthwash (oral rinse) formulation of the present invention was prepared according to the following method. 7.5 g of Lecithin E-80, 625 mg of chlorhexidine diacetate, 525 mg of Tween-80, 250 mg of D,L-Menthol and 30 mg of alpha-tocopherol acid succinate were dissolved in mixture of 20 ml of absolute ethyl alcohol and 10 ml of propylene glycol. The resultant stock solution was mixed with vigorous stirring with 480 ml of distilled water and 10 g of pure

glycerol was added as sweetener to obtain a suspension. The suspension was then filtered sequentially first through a 0.45 micron and then through a 0.22 micron PTFE membrane.

Example 6. Triclosan mouthwash formulation

300 mg of triclosan (1.05 mmol), 2000 mg (2.7 mmol) of phosphatidylcholine, 500 mg (1.7 mmol) of SLS, 300 mg of D,L-Menthol and 42 mg of aspartame were dissolved in 20 ml of absolute ethyl alcohol with slight heating (40 °C). After dissolution, 98 ml of purified water containing 20 mg of EDTA-Na (ethylenediamine tetraacetic acid sodium salt) was added slowly with vigorous stirring. The coarse suspension was treated with a high pressure homogenizer (6 cycles at 800-900 bar, 12000-14000 psi) and then filtered through a 0.22 micron PTFE membrane filter.

About 95% of the total triclosan was found to be associated with lipid particles having a mean size of about 170 nm. The antiseptic activity was unchanged.

Example 7: Non-medicated colloidal composition for evaluation of bioadhesive behavior in the oral cavity

315 mg of pure phosphatidylcholine and 80 mg of polyoxyethylated sorbitan monolaurate (Tween-20) were dissolved in 2 ml of ethyl alcohol to form a solution. The solution was diluted with purified water to a final volume of 100 ml and then passed through a 0.22 micron PTFE membrane filter. The resultant colloidal carrier had a mean droplet size of about 185 nm.

The bioadhesive properties were examined according to the following method, using the radioactive Tc^{99} label, which is safe and approved for human use. The lipid colloidal particles were labeled with Tc^{99} by using potassium pertechnate-Tc^{99}, after reduction by Sn^{2+} so that substantially all radioactivity was completely associated with lipid aggregates. A water solution of Tc^{99} complexed

with DTPA (Diethylenetriamine pentaacetic acid), in which all radioactivity was in the aqueous phase, was used as a control. 10 ml of either the labeled colloidal composition or the control solution was administered to the oral cavity of the volunteer human subject, and was then expectorated by the subject after a short rinse. As shown in Figure 1, more than 20% of the radioactive label associated with the colloidal carrier remained attached to gum and palate tissues over 2.5 hours after expectoration. By contrast, the radioactive label level for the control water solution dropped below 20% of its initial value after less than 20 minutes following rinse, and the remaining radioactivity detected was extremely low after this time.

Example 8. Chlorhexidine colloidal self-emulsifying antiseptic composition

450 mg (0.6 mmol) of purified egg lecithin, 150mg (0.25 mmol) of chlorhexidine diacetate, 150 mg of PEG-10 laurate and 450 mg (0.5 mmol) of triolein were all mixed together and heated to 60°C for 20 minutes until dissolution. Water was then added to this solution with gentle stirring. Immediately, a fine oil-in-water emulsion was formed. Such emulsions were observed to be stable with final oil phase concentrations of 5% – 25%. The resultant emulsion can optionally be treated by sonication, extrusion or high-pressure homogenization to standardize the size of emulsion droplets.

Example 9. Triclosan colloidal self-emulsifying antiseptic composition

A self-emulsifying composition containing 0.03 - 0.2% triclosan was prepared as described in example 8, except that triclosan was used instead of chlorhexidine diacetate, and 150 mg of Tyloxapol was added instead of PEG-10 laurate. After formation of the emulsion, the mixture was treated by high-pressure homogenization (6 cycles, 800 bar), producing a stable emulsion.

It will be appreciated that the above descriptions are intended only to serve as examples, and that many other embodiments are possible within the spirit and the scope of the present invention.

WHAT IS CLAIMED:

1. A formulation for application to a mucosal tissue selected from the group consisting of nasal, ophthalmic, oral cavity, gastrointestinal, respiratory, vaginal and rectal, the formulation comprising:

(a) a biologically active agent selected from the group consisting of antibiotic, antiviral agent, antifungal agent, disinfectant, nutrient, anti-inflammatory agent, local anesthetic and essential oil; and

(b) a lipid carrier, said lipid carrier including at least one lipid selected from the group of amphiphilic phospholipids consisting of yolk lecithin, Soya lecithin, phosphatidylglycerol and analogs thereof, said lipid being characterized as a colloidal micellar dispersion or as an emulsion of lipid droplets dispersed in an aqueous medium, and said lipid and said agent being present in a ratio of from about 10:1 to about 1:10, such that said agent is carried by said lipid of said lipid carrier and said agent is released from said lipid in a sustained manner and over a prolonged period of time when compared to the same formulation without said at least one lipid, and such that said lipid carrier has a property of high adhesion to the mucosal tissue.

2. The formulation of claim 1, wherein said antibiotic is selected from the group consisting of erythromycin, tetracycline, and chloramphenicol.

3. The formulation of claim 1, wherein said antiviral agent is selected from the group consisting of azothymidin, acyclovir, dideoxyuridine and amantadine.

4. The formulation of claim 1, wherein said antifungal agent is selected from the group consisting of ketoconazole, fluconazole, miconazole, tolnaftate, amphotericin and nystatin.

5. The formulation of claim 1, wherein said disinfectant is selected from the group consisting of chlorhexidine and salts thereof, triclosan, cetrimide and cetylpyridinium chloride.

6. The formulation of claim 1, wherein said nutrient is selected from the group consisting of vitamin A, vitamin E, vitamin D, vitamin K, ascorbyl palmitate, coenzyme Q-10, coenzyme Q-50, lipoic, biotin and carnitine.

7. The formulation of claim 1, wherein said anti-inflammatory agent is selected from the group consisting of non-steroidal and steroidal.

8. The formulation of claim 7, wherein said non-steroidal anti-inflammatory agent is selected from the group consisting of indomethacin, ketoprofen, diclofenol and acetylsalicylic acid.

9. The formulation of claim 7, wherein said steroidal anti-inflammatory agent is selected from the group consisting of dexamethazone, prednisolone and fluoromethzolone acetonide.

10. The formulation of claim 1, wherein said local anesthetic is selected from the group consisting of lidocaine, trimecaine and benzocaine.

11. The formulation of claim 1, wherein said essential oil is selected from the group consisting of menthol, vanillin, peppermint oil, clove oil, eucalyptus oil and lavender oil.

12. The formulation of claim 1, wherein said agent is further characterized by having activity in the oral cavity for treatment of at least one condition selected from the group consisting of gum disease, caries, dry mouth, malodorous breath, and microbial infection.

13. The formulation of claim 12, wherein said microbial infection includes an infection selected from the group consisting of bacterial, viral and fungal.

14. The formulation of claim 1, wherein said agent is further characterized by having activity on a tissue selected from the group consisting of nasal, ophthalmic, vaginal and rectal, said activity being suitable for treatment of

at least one condition selected from the group consisting of inflammation, irritation, dryness and microbial infection.

15. The formulation of claim 14, wherein said microbial infection includes an infection selected from the group consisting of bacterial, viral and fungal.

16. The formulation of claim 1, wherein said lipid and said agent are present in a ratio of from about 5:1 to about 1:5.

17. The formulation of claim 16, wherein said lipid and said agent are present in a ratio of from about 3:1 to about 1:3.

18. The formulation of claim 1, further comprising a stabilizer, said stabilizer including at least one surfactant selected from the group consisting of non-ionic, anionic, cationic and amphiphilic.

19. The formulation of claim 18, wherein said stabilizer is non-ionic surfactant selected from the group consisting of a polyethylene glycol derivatives and glycerol derivatives.

20. The formulation of claim 19, wherein said polyethylene glycol derivative is selected from the group consisting of Tweens, tritons, tyloxapol, pluronics, Brijes, Spans, poloxamers and emulphors.

21. The formulation of claim 19, wherein said glycerol derivative is selected from the group consisting of polyglycerines and polyalkylglycerides.

22. The formulation of claim 18, wherein said stabilizer is an anionic surfactant selected from the group consisting of carboxylates, alkyl and aryl sulphonates and phosphates.

23. The formulation of claim 18, wherein said stabilizer is a cationic surfactant selected from the group consisting of alkyl pyridinium salt and tetra-alkylammonium salt.

24. The formulation of claim 18, wherein said stabilizer is an amphiphilic surfactant selected from the group consisting of alkyl betaine

derivatives, cocoamphodiacetale derivatives, lauroamphoacetates and phosphatidylglycerol.

25. The formulation of claim 1, further comprising at least one lipid additive selected from the group consisting of triglycerides, alkyl esters, cholesterol, triolein, edible oils, medium chain glycerates, isopropylmyristate and cholesterol esters.

26. The formulation of claim 1, further comprising at least one additive selected from the group consisting of flavors, aroma modifiers, sweeteners, colors, and antioxidants.

27. The formulation of claim 1, wherein said lipid is in a colloidal dispersion of a form selected from the group consisting of micelles, mixed micelles and micellar aggregates, said lipid having a particle size of from about 10 to about 300 nm.

28. The formulation of claim 1, wherein said lipid is in the form of an dispersion having lipid particles of size in the range of from about 50 to about 500 nm.

29. A method of administering a formulation to a mucosal tissue selected from the group consisting of nasal, ophthalmic, oral cavity, gastrointestinal, respiratory, vaginal and rectal, comprising the steps of:

(a) providing the formulation, the formulation featuring:

(i) a biologically active agent selected from the group consisting of antibiotic, antiviral agent, antifungal agent, disinfectant, nutrient, anti-inflammatory agent, local anesthetic and essential oil; and

(ii) a lipid carrier, said lipid carrier including at least one lipid selected from the group of amphiphilic phospholipids consisting of yolk lecithin, Soya lecithin, phosphatidylglycerol and analogs thereof, said lipid being characterized as a colloidal micellar dispersion or as an

emulsion of lipid droplets dispersed in an aqueous medium, and said lipid and said agent being present in a ratio of from about 10:1 to about 1:10, such that said agent is carried by said lipid of said lipid carrier and said agent is released from said lipid in a sustained manner and over a prolonged period of time when compared to the same formulation without said at least one lipid, and such that said lipid carrier has a property of high adhesion to the mucosal tissue; and

(b) administering the formulation to the mucosal tissue.

30. The method of claim 29, wherein the mucosal tissue is the oral cavity and the formulation is administered as a mouthwash.

AMENDED CLAIMS
[received by the International Bureau on 15 March 1999 (15.03.99.); original claims 1-30 replaced by new claims 1-30 (4 pages)]

1. A formulation for topical application to a mucosal tissue selected from the group consisting of nasal, ophthalmic, oral cavity, gastrointestinal, respiratory, vaginal and rectal, the formulation comprising:

(a) a biologically active agent selected from the group consisting of antibiotic, antiviral agent, antifungal agent, disinfectant, nutrient, anti-inflammatory agent, local anesthetic and essential oil; and

(b) a lipid carrier, said lipid carrier including at least one lipid selected from the group of amphiphilic phospholipids consisting of yolk lecithin, Soya lecithin, phosphatidylglycerol and analogs thereof, said lipid being characterized as a colloidal micellar dispersion of a particle size of less than about 200 nm, such that said lipid carrier has a property of high adhesion to the mucosal tissue, and said lipid and said agent being present in a ratio of from about 10:1 to about 1:10, said lipid and said agent forming mixed micelles, such that said agent is carried by said lipid of said lipid carrier and said agent is released from said lipid in a sustained manner and over a prolonged period of time when compared to the same formulation without said at least one lipid.

2. The formulation of claim 1, wherein said antibiotic is selected from the group consisting of erythromycin, tetracycline, and chloramphenicol.

3. The formulation of claim 1, wherein said antiviral agent is selected from the group consisting of azothymidin, acyclovir, dideoxyuridine and amantadine.

4. The formulation of claim 1, wherein said antifungal agent is selected from the group consisting of ketoconazole, fluconazole, miconazole, tolnaftate, amphotericin and nystatin.

5. The formulation of claim 1, wherein said disinfectant is selected from the group consisting of chlorhexidine and salts thereof, triclosan, cetrimide and cetylpyridinium chloride.

6. The formulation of claim 1, wherein said nutrient is selected from the group consisting of vitamin A, vitamin E, vitamin D, vitamin K, ascorbyl palmitate, coenzyme Q-10, coenzyme Q-50, lipoic, biotin and carnitine.

7. The formulation of claim 1, wherein said anti-inflammatory agent is selected from the group consisting of non-steroidal and steroidal.

8. The formulation of claim 7, wherein said non-steroidal anti-inflammatory agent is selected from the group consisting of indomethacin, ketoprofen, diclofenol and acetylsalicylic acid.

9. The formulation of claim 7, wherein said steroidal anti-inflammatory agent is selected from the group consisting of dexamethazone, prednisolone and fluoromethzolone acetonide.

10. The formulation of claim 1, wherein said local anesthetic is selected from the group consisting of lidocaine, trimecaine and benzocaine.

11. The formulation of claim 1, wherein said essential oil is selected from the group consisting of menthol, vanillin, peppermint oil, clove oil, eucalyptus oil and lavender oil.

12. The formulation of claim 1, wherein said agent is further characterized by having activity in the oral cavity for treatment of at least one condition selected from the group consisting of gum disease, caries, dry mouth, malodorous breath, and microbial infection.

13. The formulation of claim 12, wherein said microbial infection includes an infection selected from the group consisting of bacterial, viral and fungal.

14. The formulation of claim 1, wherein said agent is further characterized by having activity on a tissue selected from the group consisting of nasal, ophthalmic, vaginal and rectal, said activity being suitable for treatment of at least one condition selected from the group consisting of inflammation, irritation, dryness and microbial infection.

15. The formulation of claim 14, wherein said microbial infection includes an infection selected from the group consisting of bacterial, viral and fungal.

16. The formulation of claim 1, wherein said lipid and said agent are present in a ratio of from about 5:1 to about 1:5.

17. The formulation of claim 16, wherein said lipid and said agent are present in a ratio of from about 3:1 to about 1:3.

18. The formulation of claim 1, further comprising a stabilizer, said stabilizer including at least one surfactant selected from the group consisting of non-ionic, anionic, cationic and amphiphilic, said stabilizer, said lipid and said agent forming said mixed micelles.

19. The formulation of claim 18, wherein said stabilizer is non-ionic surfactant selected from the group consisting of a polyethylene glycol derivatives and glycerol derivatives.

20. The formulation of claim 19, wherein said polyethylene glycol derivative is selected from the group consisting of Tweens, tritons, tyloxapol, pluronics, Brijes, Spans, poloxamers and emulphors.

21. The formulation of claim 19, wherein said glycerol derivative is selected from the group consisting of polyglycerines and polyalkylglycerides.

22. The formulation of claim 18, wherein said stabilizer is an anionic surfactant selected from the group consisting of carboxylates, alkyl and aryl sulphonates and phosphates.

23. The formulation of claim 18, wherein said stabilizer is a cationic surfactant selected from the group consisting of alkyl pyridinium salt and tetra-alkylammonium salt.

24. The formulation of claim 18, wherein said stabilizer is an amphiphilic surfactant selected from the group consisting of alkyl betaine derivatives, cocoamphodiacetale derivatives, lauroamphoacetates and phosphatidylglycerol.

25. The formulation of claim 1, further comprising at least one lipid additive selected from the group consisting of triglycerides, alkyl esters, cholesterol, triolein, edible oils, medium chain glycerates, isopropylmyristate and cholesterol esters.

26. The formulation of claim 1, further comprising at least one additive selected from the group consisting of flavors, aroma modifiers, sweeteners, colors, and antioxidants.

27. The formulation of claim 1, wherein said lipid has a particle size of from about 10 to about 100 nm.

28. The formulation of claim 1, wherein said lipid has lipid particles of a size in the range of from about 50 to about 200 nm.

29. A method of topically administering a formulation to a mucosal tissue selected from the group consisting of nasal, ophthalmic, oral cavity, gastrointestinal, respiratory, vaginal and rectal, comprising the steps of:

(a) providing the formulation, the formulation featuring:

(i) a biologically active agent selected from the group consisting of antibiotic, antiviral agent, antifungal agent, disinfectant, nutrient, anti-inflammatory agent, local anesthetic and essential oil; and

(ii) a lipid carrier, said lipid carrier including at least one lipid selected from the group of amphiphilic phospholipids consisting of yolk lecithin, Soya lecithin, phosphatidylglycerol and analogs thereof, said lipid being characterized as a colloidal micellar dispersion of a particle size of less than about 200 nm, such that said lipid carrier has a property of high adhesion to the mucosal tissue, and said lipid and said agent being present in a ratio of from about 10:1 to about 1:10, said lipid and said agent forming mixed micelles, such that said agent is carried by said lipid of

said lipid carrier and said agent is released from said lipid in a sustained manner and over a prolonged period of time when compared to the same formulation without said at least one lipid; and

(b) topically administering the formulation to the mucosal tissue.

30. The method of claim 29, wherein the mucosal tissue is the oral cavity and the formulation is administered as a mouthwash.

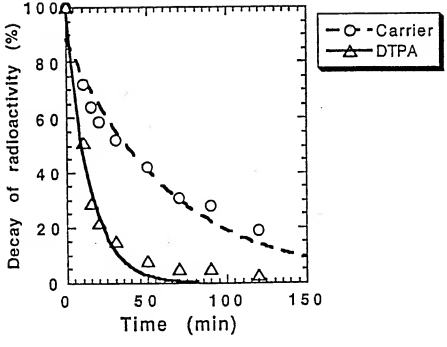
100
80
60
40
20
0
Decay of radioactivity (%)
0
50
100
150
Time (min)
Carrier
DTPA

FIG. 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL98/00504

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 7/16, 9/127, 9/107, 9/113
US CL :424/49 424/450

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/49 424/450

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,679,374 A (FANCHON et al.) 21 October 1997, lipid vesicle: lecithin, chlorhexidine, triclosan.	1 to 30
Y	US 5,662,932 A (AMSELEM et al.) 02 September 1997, phospholipid nanoemulsion emulsome: column 7.	1 to 30
Y	US 5,626,868 A (MORANCAIS et al.) 06 May 1997, lipid vesicle: chlorhexidine, triclosan, column 11.	1 to 30
Y	US 5,576,016 A (AMSELEM et al.) 19 November 1996, lecithin nanoemulsion for topical delivery.	1 to 30

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"B"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 20 JANUARY 1999	Date of mailing of the international search report 05 FEB 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer SHEP ROSE aco Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL98/00504

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,443,840 A (MORANCAIS et al.) 22 August 1995, lipid vesicles: chlorhexidine, triclosan, column 9.	1 to 30
Y	US 5,376,646 A (PITTROF et al.) 27 December 1994, topical lecithin: chlorhexidine compositions.	1 to 30
Y	US 5,128,139 A (BROWN et al.) 07 July 1992, multilamellar phospholipid liposome: triclosan.	1 to 30
Y	US 4,767,615 A (GEHO et al.) 30 August 1988, mouthwash of lecithin liposome for oral teeth and gums oral cavity medication.	1 to 30
Y	US 4,670,185 A (FUJIWARA et al.) 02 June 1987, chlurhexinine gargle vesicle dispersion.	1 to 30